NUMERIC MODELING OF CELL-CELL SIGNALING IN MICROFLUIDICS TOWARDS IN VITRO MODELS OF INTESTINAL FLORA

X.L. Luo[1*], G.W. Rubloff[2] and W.E. Bentley[2]
[1]*Catholic University of America, USA and*
[2]*University of Maryland, USA*

ABSTRACT

We report the numeric modeling of bacterial cell-cell signaling within a synthetic ecosystem, which creates a simple *in vitro* model to understand the interactions among bacterial consortia inside human intestinal flora. Naturally localized bacterial quorum sensing was remotely established by controlling the transport of AI-2, the universal cell signaling molecule between a transmitter cell population and a reporter cell population in separated microchannels. Active modulation of the remotely communicating AI-2 was numerically simulated with an engineered modulating cell community that either enhance or eliminate the cell-cell signalling between two already communicating populations. Based on the modulation effects quantified with bioassay, the reaction rates of signal production and consumption were elucidated in the numeric simulation. Distribution of signaling molecules in the cell-gel composites from numeric simulations agrees with the cellular response of experimental results. We envision this simple platform can be extended to other cell-cell interaction studies among various species or kingdoms of cells within controlled microenvironments.

KEYWORDS: Cell-Cell Signaling, Intestinal Flora, Membrane, Hydrogel

INTRODUCTION

Biological functions are often guided by signaling between cells. Inside the gut, molecules transmitted by commensal microorganisms in the digestive tract are transported longitudinally through the stomach, small intestines and large intestines, and are essential to the symbiotic relationships within the microbiome and between the microbiome and the host [1]. *In vitro* models of this incredibly complex system have drawn significant interests in recent years [2]. We have introduced "biofabrication" as a means to construct synthetic ecosystem in microfluidics for direct observation and manipulation of bacterial cell-cell signaling by controlling local microenvironment [3] and by engineered quorum sensing cell populations [4]. Here, we use numerical tools to quantitatively (1) model the cell-cell signaling events in the biofabricated synthetic ecosystems, and (2) elucidate the dynamics of the synthesis, sensing and modulation of the communicating signals among the transmitter, reporter and modulator cell populations (Fig.1). The transportation and active modulation of the communicating signal molecules in the cascaded microfluidic network represent an initial foray to tackle the complex symbiotic relationships in an *in vitro* model of the gut.

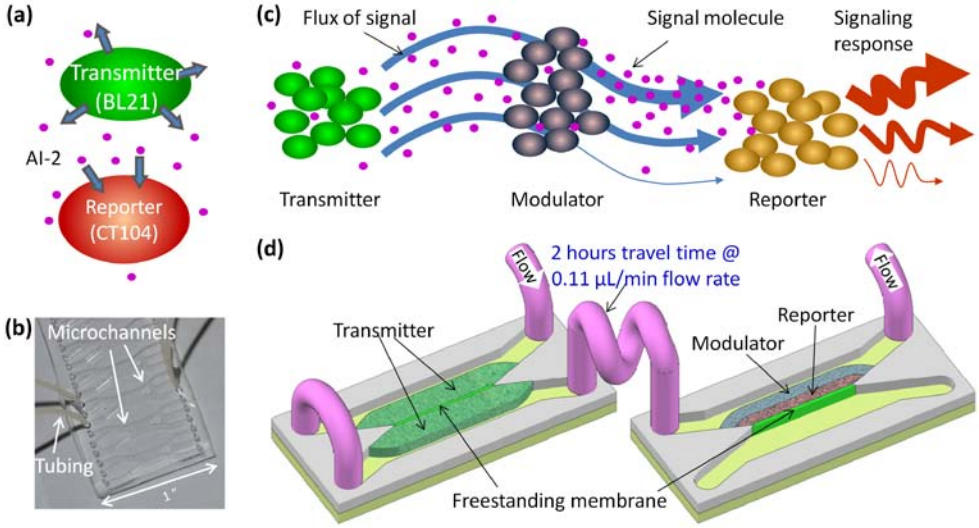

Fig.1: Scheme of bacterial cell-cell signaling mediated with quorum sensing engineering cells. ***(a)*** *Signal molecule AI-2 produced by transmitter (BL21) induces DsRed production in reporter (CT104).* ***(b)*** *A device with multiple microchannels on a 3" X 1" glass slide.* ***(c)*** *Flux of signal molecules from transmitter to reporter is either enhanced or reduced by modulating cells.* ***(d)*** *Schematic of spatially localized cell-gel composites in connected microchannels.*

MATERIALS AND METHODS

Microfluidic devices in Fig.1(b) were fabricated with soft lithography techniques. Spatially localized cell-gel composites were assembled using a previously reported biofabrication approach [2]. The two microchannels were remotely connected with a PTFE tubing that is 10 cm long to mimic the signal transport in intestinal flora. As a result, it takes 2 hours for a 0.11 µL/min flow to transport small molecules from the upstream channel to the downstream channel. Cell signaling studies were performed using an environmental chamber, the cellular responses were monitored with an

inverted fluorescent microscope, and images were processed using ImageJ. Numeric simulations were run with the software COMSOL 4.3.

RESULTS AND DISCUSSION

We first performed AI-2 activity bioassay BB170 by analyzing the effluent solutions collected after the assembled transmitter cells in the upstream channel (control) and after one assembled layer of cell-gel in the downstream channels containing either reporter, enhancer or reducer cells. The rough amount of AI-2 produced by the transmitter cells and the effect of the modulator cells on the communicating AI-2 were determined BB170 bioassay and shown in Fig. 2(a). In the numeric modeling, the reporter cell-gel was regarded as a AI-2 sink, the enhancer cell-gel was regarded as another AI-2source, while the reducer cell-gel was regarded as a big sink of AI-2 in the flow. The porosity of all the cell-gel composites was assumed to be 95 [5], and the permeability of which was assumed to be 10^{-9} cm^2 [6]. Velocity distributions of the flow along the modulating cell-gel composite were numerically computed as shown in Fig.2(b). A parameter sweeping strategy in COMSOL was applied to extract the reaction rates in the downstream cell-gel composites. The reaction rates of the transmitter, reporter, enhancer and consumer cells were determined to be 4.0 µM/s, - 0.95 µM/s, 0.37 µM/s and - 12 µM/s, respectively. The AI-2 distributions in the upstream transmitter cell-gel composite and in the downstream reporter and modulator cell-gel composites were simulated and shown in Fig.2(c).

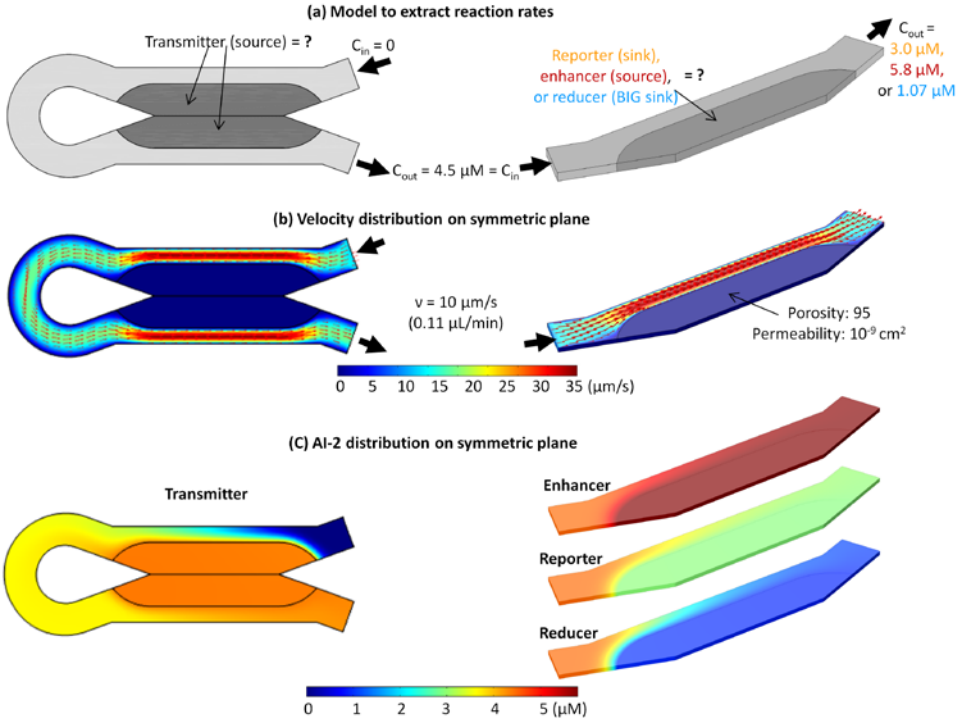

Fig.2: Models to extract reaction rates of the reporter, enhancer and reducer cells. ***(a)*** *Numeric model with measured AI-2 concentrations in flow.* ***(b)*** *Velocity distribution of the symmetric middle plane.* ***(c)*** *AI-2 distributions in transmitter, reporter, enhancer and reducer cell-gel composites.*

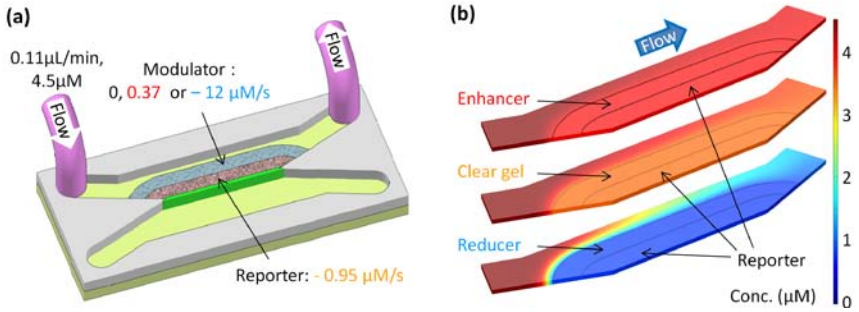

Fig.3: Simulated AI-2 distribution in cell-gel composites. ***(a)*** *Schematic with known in-flow parameters and extracted reaction rates.* ***(b)*** *AI-2 distribution on the middle planes of cell-gel composites in the cases of enhancer, clear gel (control) and one layer of reducer as modulator.*

Next, numerically quantified reaction rates of the modulating cells were used to compute the AI-2 distribution in the downstream channels where modulator and reporter cells were assembled side-by-side as shown in Fig.3(a). The results in Fig.3(b) show that AI-2 uniformly distributes throughout the reporter and modulator cell-gels with the magnitudes of

above 4 µM, around 3 µM and below 1 µM for the cases of enhancer, clear gel and one layer of reducer as the modulator, respectively. Typical optical density of cell growth in Fig.4(a) was obtained directly from bright field images taken with transmitted light. Assuming the AI-2 production/ consumption rates were constant, time-course AI-2 concentration within the reporter cell-gel composites are calculated and shown in Fig.4(b). The time-course tendency of AI-2 in Fig.4(b) agreed with the time-course curves of experimentally measured fluorescence intensity of the reporter cells in Fig.4(c) for the cases of enhancer, clear gel and one layer of reducer as modulator. Due to the immediate availability of AI-2, the reporter cellular response in the enhancer case was 2 hours ahead than that in the control and reducer cases. In all cases, there was a 4-hour delay for protein production after the availability of AI-2. Combined, the results demonstrate that the third cell populations were effective to modulate the amount of signal molecule AI-2 communicating between the transmitter and reporter cells, therefore modulating the cellular response of the reporter cells.

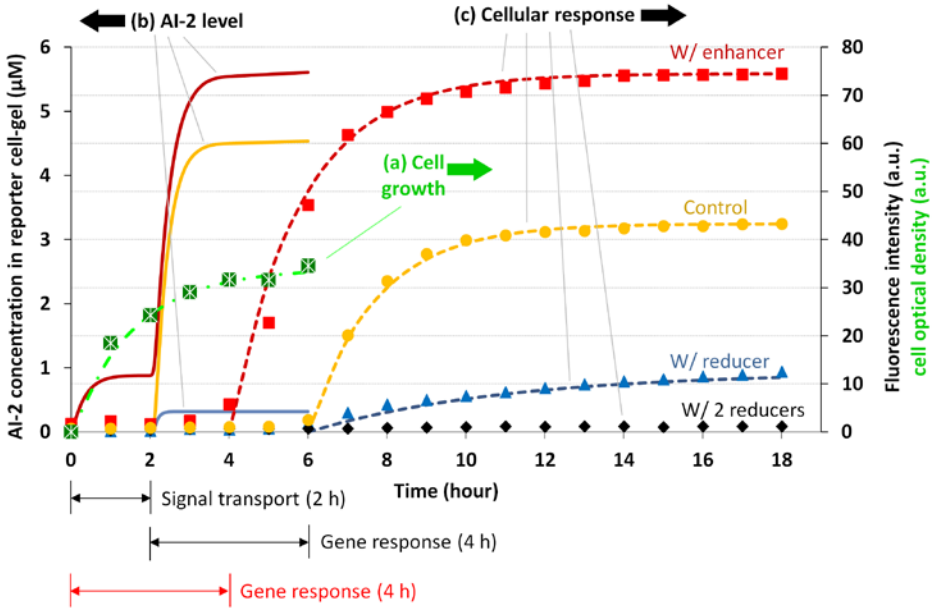

Fig.4: Time-course cellular response of reporting cells with the corresponding AI-2 concentration within the reporter cell-gel composites. ***(a)*** *Typical cell growth curve based on optical density measurement of cells.* ***(b)*** *Estimated AI-2 concentration within the reporter cell-gel composites for the beginning 6 hours for the cases of enhancer, clear gel (control) and one layer of reducer as modulator.* ***(c)*** *Time-course fluorescence intensity of the reporter cells over 18 hours for the cases of enhancer, clear gel (control), one layer of reducer, and two layers of reducer as modulator.*

CONCLUSION

Combining experimental assays and numerical simulations, we have quantitatively evaluated the synthesis, consumption and dynamics of signaling molecules with flow in cascaded microfluidic channels, and the active modulation of the signaling with the third engineered cell communities. The study offers guidance to investigate more complex *in vitro* models of the gut in microsystems.

ACKNOWLEDGEMENTS

This work was supported by the Burns Junior Faculty Fellowship at the Catholic University of America, the Robert W. Deutsch Foundation and the Defense Threat Reduction Agency (DTRA#BO08SPO008). We acknowledge the support of the Maryland NanoCenter and its FabLab.

REFERENCES

[1] Guarner F. and Malagelada J-R., *Lancet* 2003, 361, 512-519.
[2] Kim H., Huh D., Hamilton G. and Ingber D.E., *Lab on a Chip*, 2012, 12, 2165-2174.
[3] Luo X., Wu H., Tsao C., Cheng Y., Betz J., Payne G., Rubloff G. and Bentley W., *Biomaterials*, 2012, 33, 5136-5143.
[4] Luo X., Wu H., Tsao C., Rubloff G. and Bentley W., *BMES Annual Meeting*, Atlanta, GA, October 24-27, 2012.
[5] Partap S., Muthutantri A., Rehman I. U., Davis G. R. and Darr J. A., *Journal of Materials Science*, 2007, 42, 3502-3507.
[6] Hwang C. M., Sant S., Masaeli M., Kachouie N. N., Zamanian B., Lee S.-H. and Khademhosseini A., *Biofabrication*, 2010, 2(3):035003

CONTACT

*X.L. Luo, tel: +1-202-319-6952; luox@cua.edu